STANDARD OPERATING PROCEDURE			
Instrument Quality Control			
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1. Policy

1.1. The Flow Cytometry and Cell Sorting Facility, hereafter called "FCCSF," has five instruments that require regular quality control (QC) procedures. These instruments and corresponding frequency of QC are listed below:

Instrument	Frequency of QC
BD Fortessa X-20	Every day the instrument is in use. Once weekly even if no instrument use is scheduled.
Cytek Aurora 5L	Every day the instrument is in use. Once weekly even if no instrument use is scheduled.
BDFACS Aria III	Every day the instrument is in use. Once weekly even if no instrument use is scheduled.
Vi-Cell XR Cell Analyzer	Once every two weeks
Curiox HT2000 Cell Washer	Every day the instrument is in use. Once weekly even if no instrument use is scheduled.

The FCCSF policy is to perform QC on the instruments listed above, at the frequency listed above. The Seahorse XFe96 Analyzer and the Miltenyi gentleMACS Octo Dissociator and MACSQuant Tyto do not require regular QC.

2. Purpose

2.1. The FCCSF has instruments that require regular quality control (QC) procedures. These QC procedures:

- Define and characterize baseline performance
- Optimize, standardize and track cytometer performance
- Provide consistent, reproducible data
- Offset day-to-day instrument variability
- Allow for early identification of degrading instrument performance

In short, the QC procedures outlined here ensure that users of FCCSF can be confident in the validity of data generated with FCCSF instruments.

3. Responsibilities

3.1. FCCSF Manager or trained users will run QC on all instruments listed in Section 1.1, at the frequency listed in Section 1.1. The FCCSF Manager will ensure that QC is completed.

4. Procedure

4.1. BD Fortessa X-20

4.1.1. Preparation

4.1.1.1. Turn on instrument - Press the green button on the side of the instrument to turn on. Allow instrument (lasers) to warm up for 20 min.

4.1.1.2. Empty waste and fill sheath if needed.

4.1.1.3. Turn on computer if needed. Click CTRL ALT Delete to log in to the computer. Log in as BDOperator Type in password . Alternate login is BDAdmin with password . Note: Diva software can only be open in one login at a time.

4.1.1.4. Open Diva software and log in as admin.

4.1.1.5. Prepare CS&T beads - Current lot of CS&T beads is posted on a sticky note on Fortessa computer. Beads are located in the FCCSF refrigerator. Vortex beads. Add one drop of current lot number BD CS&T Research beads to 500µL PBS in a flow tube. If running CS&T using HTS, add one drop of CS&T beads and 150µL PBS to well A1 of 96-well plate.

4.1.2. Run CS&T

4.1.3. Verify that cytometer is in the correct configuration.

4.1.3.1. In top toolbar in Diva workspace, go to "***Cytometer>CST>view configuration.***" The appropriate configuration for tubes is "***CST Texas A&M X-20 2-Blue 3-Red 6 Violet 5-YG".*** The appropriate

configuration for the HTS is ***"HTS A&M X-20 2-Blue 3-Red 6 Violet 5-YG."***

4.1.3.2. To change the configuration, select appropriate configuration, click **"Set Configuration"** and close out of the configuration window.

4.1.4. In top toolbar in Diva workspace, select "***Cytometer>CST."*** The CS&T workspace will open. Confirm that ***"Load tube manually"*** is checked if running tubes and unchecked for running in plate.

4.1.5. Verify that the bead lot ID selected matches your current lot of CS&T beads.

4.1.6. Check that the cytometer baseline has been defined and is valid. If not, ask FCCSF manager to define a new baseline.

4.1.7. Press the "***Run"*** and "***Low"*** buttons on fluidics panel

4.1.8. Load the tube of CS&T beads on the SIP if running tubes. For HTS, load the plate on the HTS.

4.1.9. In the Setup Control window, select "***Check Performance"*** and click "***Run"***.

CS&T application will automatically run. This will take approximately 5 -8 minutes.

4.1.10. Review results

4.1.11. At the end of Check Performance, CS&T will either Pass, Pass with Warnings or Fail.

4.1.11.1. If CS&T passes, close the "***Setup Control"*** window to return to Diva browser. The instrument is ready for use. FCCSF manager monitors Levey-Jennings plots and Reports for any trends.

4.1.11.2. CS&T will pass with warnings if any of the primary channel rCVs are greater than 6. In this case, run a cleaning procedure (ref. 5.1) and re-run CS&T. If CS&T passes with warnings again, report to FCCSF manager and run experiment. FCCSF manager will clean the flow cell and check for any other potential causes.

4.1.11.3. CS&T will fail if PMT voltage changes more than by more than 50 to reach target value in any detector. CST will not run if it there are not enough events to proceed. CS&T will not be able to put beads on target if there are air bubbles in the flow cell.

4.1.11.3.1. If CS&T fails, remake beads, run in an experiment and make sure CS&T beads have three peaks in every fluorescent channel. If beads look as expected, run CS&T again. If CS&T fails again, run cleaning (ref 5.2) procedure and repeat. If CS&T fails again, report to FCCSF manager. If issue not resolved after troubleshooting, call for service. Take instrument offline and alert users.

4.1.11.3.2. If CS&T will not run because there are not enough events to proceed, remake beads and repeat. If error message persists, run cleaning procedure and repeat. If further problems are encountered, alert FCCSF manager.

4.1.11.3.3. If error message “Cannot put beads on target” appears, prime three to five times and run PBS for 10 min and repeat CS&T. If error persists, notify FCCSF Manager.

4.1.11.3.4. NOTE: Cytometer may be used at user’s discretion if CST fails due to FSC or SSC PMTV>50 or if the affected parameter is not included in the user’s experiment.

4.2. Cytek Aurora 5L

4.2.1. Preparation

4.2.1.1. Turn on instrument - Press the button on the side of the instrument to turn on. Allow instrument (lasers) to warm up for 30 min.

4.2.1.2. Open SpectroFlo software and log in to your user profile.

4.2.1.3. Empty waste and fill sheath if needed. Run MQ water at high flow rate for 30 min while lasers are warming up. Return flow rate to low after 30 min.

4.2.1.4. Prepare QC beads - Beads are located in the FCCSF refrigerator. Add one drop of current lot number SpectroFlo QC beads to 300µL MQ water in a flow tube or plate well. QC beads must be prepared in the same solution used for the sheath solution, therefore MQ water must be used. Beads will begin to degrade within a few hours. Do not reuse beads prepared in water.

4.2.2. Run QC

4.2.2.1. Select ***QC & Setup*** from the ***Get Started*** menu.

4.2.2.2. Verify that the bead lot ID selected matches your current lot of QC beads. Each time you open a new lot number of SpectroFlo QC beads, you must import the bead lot ID into the library, so it is accessible when you run QC. Bead lot files can be downloaded from the Resources section at http://www.cytekbio.com

4.2.2.3. Ensure the correct Carrier Type is selected

4.2.2.3.1. Tube: Load a 12x75-mm tube of the beads onto the SIP. Select ***Start*** to begin acquisition.

4.2.2.3.2. Loader: Ensure the correct plate type is selected. Click in the plate to select the well where the QC beads are located. Well A1 is selected by default, but you can choose any well. Select ***Eject***, load the plate onto the plate stage, then click ***Load*** followed by ***Start*** to begin acquisition.

4.2.2.3.3. As the instrument begins acquiring the QC beads, they appear in the scatter plot. The procedure takes approximately 3 -5 minutes. The performance measurements are established and compared to pass/fail criteria.

4.2.3. Review results

4.2.3.1. At the end the procedure, QC will either pass or fail.

4.2.3.1.1. If QC passes, return to ***Acquisition*** and run your samples. The instrument is ready for use. FCCSF manager monitors Levey-Jennings plots and reports for any trends.

4.2.3.1.2. Pass/Fail Criteria

4.2.3.1.2.1. %rCV must not exceed 6% for the FSC channel

4.2.3.1.2.2. %rCV must not exceed 8% for the SSC and SSC-B channels

4.2.3.1.2.3. %rCV must not exceed 6% for the third channel of each laser (V3, B3, R3, YG3, and UV3)

4.2.3.1.2.4. % delta gain change for all channels must not exceed 100% from the last Daily QC run performed by Cytek Service personnel.

4.2.3.1.2.5. If QC fails, remake beads, run in an experiment and make sure QC beads have signal in every fluorescent channel. If beads look as expected, run QC again. If QC fails again, run ***Clean Flow Cell*** procedure, so several SIT flushes. If QC fails again, report to FCCSF manager. If issue not resolved after troubleshooting, call for service. Take instrument offline and alert users.

4.3. BD FACS Aria III

4.3.1. Preparation

4.3.1.1. Turn on instrument - Press the green button on the side of the instrument to turn on. Allow instrument (lasers) to warm up for 20 min.

4.3.1.2. Empty waste and fill sheath if needed.

4.3.1.3. Turn on computer if needed. Click CTRL ALT Delete to log in to the computer. Log in as BDAdmin. Type in password BDIS#1.

4.3.1.4. Open Diva software and log in to your user profile. Perform fluidics startup. Insert correct nozzle.

4.3.1.5. Prepare CS&T beads - Current lot of CS&T beads is posted on a sticky note on Aria computer. Beads are located in the FCCSF refrigerator. Add one drop of current lot number BD CS&T Research beads to 500 µL PBS in a flow tube.

4.3.2. Run CS&T

4.3.2.1. Verify that cytometer is in the correct configuration for nozzle being used. In top toolbar in Diva workspace, go to "***Cytometer>CST>view configuration.***" Each nozzle has its own configuration. Use configuration **85-45** for 85µm nozzle, **100** for 100µm nozzle, **130** for 130µm nozzle and **70 11112015** for 70µm nozzle. Do not use any of the configurations that include "FSC PMT" without consulting FCCSF Manager. These configurations utilize the FSC PMT for detection of small particles.

4.3.2.2. To change the configuration, select appropriate configuration, click **"Set Configuration"** and close out of the configuration window.

4.3.2.3. Start stream. Make sure Flow Rate is set to 1. Wait for stream to stabilize.

4.3.2.4. In top toolbar in Diva workspace, select "***Cytometer>CST."*** The CS&T workspace will open.

4.3.2.5. Verify that the bead lot ID selected matches your current lot of CS&T beads.

4.3.2.6. Check that the cytometer baseline has been defined and is valid. If not, ask FCCSF manager to define a new baseline.

4.3.2.7. Load the tube of CS&T beads into the sample chamber.

4.3.2.8. In the Setup Control window, select "***Check Performance"*** and click "***Run"***. CS&T application will automatically run. This will take approximately 5-8 minutes.

4.3.3. Review results

4.3.3.1. At the end of Check Performance, CS&T will either Pass, Pass with Warnings or Fail.

4.3.3.2. If CS&T passes, close the "***Setup Control"*** window to return to Diva browser. The instrument is ready for use. FCCSF manager monitors Levey-Jennings plots and Reports for any trends.

4.3.3.3. CS&T will pass with warnings if any of the primary channel rCVs are greater than 6. In this case, run a cleaning procedure (10% bleach 5 min, 1.5% Citranox 5 min then DI water for 5 min) and re-run CS&T. If CS&T passes with warnings again, report to FCCSF manager and run experiment. FCCSF manager will clean the flow cell, further troubleshoot and call service if needed.

4.3.3.4. CS&T will fail if PMT voltage changes more than by more than 50 to reach target value in any detector. CST will not run if it there are not enough events to proceed. CS&T will not be able to put beads on target if there are air bubbles in the flow cell.

4.3.3.4.1. If CS&T fails, remake beads, run in an experiment and make sure CS&T beads have three peaks in every fluorescent channel. If beads look as expected, run CS&T again. If CS&T fails again, run cleaning procedure and repeat. If CS&T fails again, report to FCCSF manager. If issue not resolved after troubleshooting, call for service. Take instrument offline and alert users.

4.3.3.4.2. If CS&T will not run because there are not enough events to proceed, remake beads and repeat. If error message persists, run cleaning procedure and repeat. If further problems are encountered, alert FCCSF manager.

4.3.3.4.3. If error message "Cannot put beads on target" appears, run PBS for 10 min and repeat CS&T. If error persists, notify FCCSF Manager.

4.3.3.4.4. NOTE: Cytometer may be used at user's discretion if CST fails due to FSC or SSC PMTV>50 or if the affected parameter is not included in the user's experiment.

4.4. Vi-Cell XR Cell Analyzer

4.4.1. Preparation

4.4.1.1. Beckman Coulter Vi-CELL Concentration Control is stored in the FCCSF refrigerator. Remove from refrigerator and vortex Vi-Cell Concentration Control for 10-12 seconds to ensure proper mixing.

4.4.1.2. Place 600ml of control into a sample cup.

4.4.1.3. Place the sample on the sample carousel.

4.4.2. Run QC

4.4.2.1. From the navigation bar, select ***Concentration Control***

4.4.2.2. Double click on the control icon and assign a position in the ***Login*** dialog box. Click ***OK***.

4.4.2.3. Make certain cell type is correct.

4.4.2.4. Select ***Start.***

4.4.3. Review results

4.4.3.1. Check the current results window for statistics. The total cells/ml should be within ± 10% Assay Value as listed in the Assay Sheet for the Concentration Control (somewhere close to 1x10^6/ml).

4.4.3.2. Record value in QC log.

4.4.3.3. If value fails to fall within the passing criteria described in 4.4.3.1 above, remake control being sure to mix well. If that doesn't work, try a new bottle. If it still fails, notify FCCSF manager to call service.

4.4.3.4. Note: Do not freeze concentration control. Freezing results in increased counts. Do not leave bottle uncovered for extended periods of time. Control is guaranteed to be stable at 2-8°C until the expiration date or 30 days after opening and storing at 2-8°C.

4.5. Curiox HT2000 Cell Washer

4.5.1. Preparation

4.5.1.1. QC of the Curiox is included in the beginning-of-day priming/calibration protocol that users must carry out prior to beginning wash. Immediately after priming the Curiox (with the system still loaded with DI water + 1% tween20), replace the dummy plate with the calibration plate. Perform once a week even if no experiments are scheduled.

4.5.2. Run QC

4.5.2.1. In SERVICE MODE → press DISPENSE 80uL (on screen 2/3) – once ready, remove the plate from the feeder – check visually that the volume in all the wells are even (80 uL) – put the plate back on the feeder.

4.5.2.2. Switch to OPERATION MODE.

4.5.2.3. Adjust parameters: 10 uL/s flow rate, 80 uL initial volume, and 4 wash cycles.

4.5.2.4. Press START to perform a wash – once it's ready, remove the plate from the feeder.

4.5.3. Review results

4.5.3.1. Check visually that the leftover volume in all the wells is even. Verification of this passing criteria ensures that the Curiox is working correctly and will properly achieve laminar flow and washing.

4.5.3.2. If volume is uneven, please alert the FCCSF manager immediately for assistance with troubleshooting.

5. References

5.1. BD Fortessa X-20 QC Procedure

5.1.1. Using the BD Cytometer Setup and Tracking (CS&T) System for Instrument Characterization and Performance Tracking; Mark KuKuruga, Senior Technical Applications Specialist, BD Biosciences

5.1.2. FCCSF After-Hours Training PowerPoint (Appendix)

5.1.3. BD Cytometer Setup and Tracking Application Guide for BD FACS Digital Flow Cytometers (Appendix)

5.2. Cytek Aurora 5L QC Procedure

5.2.1. Cytek User Manual Revision N9-20006 Rev. B 10/2019

5.2.2. Cytek Video Tutorial: Reference Control QC Tools

5.2.3. Cytek Video Tutorial: Bubble and Clog Detection

5.3. BD FACS Aria III QC Procedure

5.3.1. Using the BD Cytometer Setup and Tracking (CS&T) System for Instrument Characterization and Performance Tracking; Mark KuKuruga, Senior Technical Applications Specialist, BD Biosciences

5.3.2. FCCSF Aria Cleaning and Shut Down (Appendix)

5.3.3. BD Cytometer Setup and Tracking Application Guide for BD FACS Digital Flow Cytometers (Appendix)

5.4. Vi-Cell XR Cell Analyzer QC Procedure

5.4.1. Beckman Coulter Particle Characterization Assay Sheet for Vi-CELL Concentration Control (Appendix)

5.4.2. Vi-CELL XR Instructions for Use Manual, 2018, pg 2-11 to 2-12. (Appendix)

5.5. Curiox HT2000 Cell Washer QC Procedure

5.5.1. FCCSF Curiox Training Info (Appendix)

6. Date established and subsequent reviews (annually at a minimum)

6.1. List the date of establishment

6.2. List dates of subsequent reviews or changes to the procedure.

Revision	Review or Revision Date	Description of Changes	Reviewed By
0.0	1/1/2022	Initial Release	LCW&RM
10	10/1/2024	Addition of Curiox	EV&RM